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Hepatoregenerative effect of *Nyctanthes arbortristis* Linn. on acetaminophen induced oxidative damage in rats

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Abstract: Initiation of acetaminophen (APAP) toxicities is believed to be promoted by oxidative stress during the event of overdosage.The aim of the present study was to evaluate the hepatoregenerative effect of Nyctanthes arbortristis (NAT) was evaluated by intoxicating the rats with 1g/kg of acetaminophen(APAP) *p.o.* for seven days. The Methanolic extract of the leaves was administered at 3 standard dose levels like 250mg/kg, 500mg/kg and 750mg/kg for 9 days after the third dose of acetaminophen. The hepatoprotective activity of NAT extract was observed following significant histopathological analysis and reduction of the level of Alanine aminotransferase (ALT) Aspartate aminotransferase (AST) and Total bilirubin (TB) in groups treated with NAT compared to those treated with APAP alone. Meanwhile, the level of glutathione (GSH) was found to be restored in NAT treated animals compared to the groups treated with APAP alone. These observations were comparable to the group treated with Silymarin. Group that was treated with APAP alone exhibited high level of transaminases besides reduction in the GSH level. Histological hepatocellular deterioration were also evidenced. The results from the present study suggested that the leaves of NAT can prevent hepatic injuries from APAP induced through preventing the decline of glutathione level.
Key words: *Nyctanthes arbortristis*, acetaminophen, Hepatoregenerative.

Introduction

Liver diseases have become a major stumbling block to twentieth century medicine. Capacity for regeneration of the liver is considerable and damage is usually extensive before it is evident. The effects of liver disease are seen when; regeneration of hepatocytes does not keep pace with damage leading to hepatocellular failure. Acetaminophen (APAP) is an effective and widely used antipyretic- analgesic drug with excellent safety record when taken at therapeutic doses. Overdose of APAP results in the generation of free radicals following the depletion of glutathione.[1] Numerous medicinal plants and their formulation are used for liver disorders in ethnomedical practice.[2,10] *Nyctanthes arbortristis* (NAT) is commonly known as Harsinghar, Prajakta or Night Jasmine. Leaves of *Nyctanthes arbortristis* contain irridoid glucosides, an alkaloidal principle named nyctanthine, mannitol, astringent principles, resinous substances, ascorbic acid.[3] Plant is cholagogue and laxative. Its extract is used in menorrhagia, sores and ulcers, rheumatism, chronic fever, in loss of appetite, piles and biliary disorders.[3] There are scanty reports on the putative hepatoprotective potential of NAT. Therefore, the objective of this study is to evaluate the hepatoregenerative potential of methanol extract of NAT against hepatotoxicity induced by APAP. The hepatoregenerative potential of the NAT extract was compared with silymarin, a known and commercially available hepatoprotective agent.[1,11]

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Materials and methods

Plant Material and reagents

Nyctanthes arbortristis leaves were obtained from local source college campus and were authenticated by Dr. Harshad Pandit, G.N.Khalsa College Mumbai. A voucher specimen (2009/03/10) has been kept in our departmental herbarium for future reference.

Chemicals and Instruments:

Acetaminophen was obtained from NuLife pharmaceuticals .Silymarin was purchased as Silybon 140 tablets and Enzymes like AST, ALT, ALP, Total bilirubin and protein were assayed using standard Merck diagnostic kits. Refrigerated Centrifuge, Superspin, Plasto-crafts, India was used to separate serum from blood.

Preparation of plant extract

The leaves were shade dried and powdered in mixer grinder and stored in tightly closed container. The dried powder of leaves of *Nyctanthes arbortristis* was first subjected to Soxhlet extraction with petroleum ether to remove fatty materials and chlorophyll pigments. The extraction was carried out at 40-60 ºC in Soxhlet extractor. The defatted powder of leaves of *Nyctanthes arbortristis* was then subjected to extraction with methanol. The extraction was carried out at 60-70ºC in Soxhlet extractor for 24 hrs. The methanolic extract was then concentrated under vacuum and the concentrate was dried in hot air oven at 40-50˚C. [3]

Experimental animals

Albino Wistar rats of either sex weighing between 150-200gm were procured from Haffkine pharmaceuticals and used for evaluation of hepatoprotective activity. Animals were housed in polypropylene cages (6 animals/cage) under hygienic conditions in the CPCSEA registered animal house (CPCSEA/87/1999) of Institute of Chemical Technology, University of Mumbai, Matunga, Mumbai. Animals were fed with standard pelleted diet procured from Chakan Oil Mills, Sangli, Maharashtra and supplied with fresh water *ad libitum*. Light cycle of 12hr light/dark was strictly followed in the animal house. The animals were allowed to acclimatize to laboratory conditions prior to commencement of the experiments. Animals used in this study were treated and cared for in accordance with the guidelines recommended by the committee for the purpose of control and supervision on experiments and the experimental protocol was approved by institutional animal ethics committee (IAEC/08-09/P-25).

Acute oral toxicity study

Acute oral toxicity study of methanolic *Nyctanthes arbortristis* was carried out in Swiss albino mice of either sex (20-22 g) according to OECD guidelines no 423. Extract at different doses up to 5000 mg/kg, *p.o.* was administered and animals were observed for behavioural changes, toxicity and mortality up to 48 hrs. [5,6]

Hepatoprotective activity:

The healthy animals were divided into six different groups comprising of 6 animals each. Group I served as vehicle control and administered orally with vehicle i.e. 0.5%w/v Na CMC only. Group II served as negative control and treated with only acetaminophen (1g/kg) for the first 7 days. Group III, IV, V served as treated groups and received acetaminophen (1g/kg) for the first 7 days. The groups III, IV, V received MNAT (Nyctanthes arbortristis methanolic extract) 250, 500 and 750mg/kg *p.o.* respectively from 4^{th} to 12^{th} day. Group VI Silymarin served as the positive control and received acetaminophen (1g/kg) for the first 7 days and Silymarin (100mg/kg) *p.o* from 4^{th} to 12^{th} day.

Autopsy:

Blood was withdrawn by puncturing the retro-orbital plexus on day 4 and day 13. Serum was separated from blood by centrifugation at 2000g for 15min and collected in centrifuge tubes for estimation of biochemical parameters in serum. Then animals were sacrificed under ether anaesthesia and abdomen was cut opened to isolate liver aseptically. The isolated liver was washed in normal saline (0.9%w/v NaCl), and part of cleaned liver was placed in formalin solution (10%v/v) for histopathology studies. The remaining part of liver was homogenized in 0.1M Tris HCl buffer, pH 7.4 (liver homogenate) and the homogenate was used for determination of Reduced Glutathione.

Statistical analysis

Data is expressed as Mean ± SEM and is statistically assessed using ANOVA followed by Dunnett's test. Results were considered to be statistically significant at $p<0.01$.

Results

Usually, the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes ALT (Alanine transaminase), AST (Aspartate transaminase) and hepatic damage leads to leakage of large quantities of enzymes into the blood circulation. This was associated by massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver.[9] The changes in hepatocytes induced by acetaminophen results in inhibition of mitochondrial respiration and generation of reactive oxygen species

.This aggravates the damage in hepatocytes resulting in membrane fragility and leakage of enzymes.[1] On Administration of APAP(1 mg/kg, *p.o.*) there was a significant ($p<0.01$) rise in serum AST, ALT, levels compared to respective control values due to hepatic cell necrosis which leads to membrane fragility and leakage of these enzymes in circulation **(Table 1)**. It also caused alterations in plasma total albumin, and total bilirubin levels **(Table 2)**. Treatment of rats with NAT significantly ($p<0.01$) inhibited the alterations in these biochemical levels by protection against membrane fragility thus decreased the leakage of marker enzymes in circulation. The results showed that a high dose of APAP has caused remarkably reduced level of cellular GSH. In the current study, GSH depletion was prevented when MNAT was administered. This may be due to enhancement of *de novo* GSH synthesis or GSH regeneration or both.[1,7] Acetaminophen treatment resulted in decreased activity of catalase in the liver which might be due to increased formation of free radicals or inactivation of enzyme by cross linking. MNAT possesses the capacity to scavenge free radicals produced due to acetaminophen induced hepatic damage, therby increasing catalase activity**.(Table3)** Histopathological studies provided supportive evidence for biochemical analysis. The normal control group of rats did not show any histological alterations in the hepatocytes. Histological sections of the liver showed similar reported changes including Centrilobular necrosis with inflammatory cell infiltration in rats treated with APAP alone. . Treatment with MNAT reduced the severity of histological lesions in liver such as minimal lymphocytic infiltration, minimal nuclear disintegration and minimal necrosis caused by acetaminophen intoxication. **(Table 4)**

Also the rats treated with Silymarin (100mg/kg) showed mild to moderate diffuse granular degeneration and very mild necrosis**. (Fig a-f)**

Table 1:

Groups	**Biochemical parameters**			
	AST (U/L)		ALT (U/L)	
	4th day	13th day	4th day	13th day
Control	52.00 ± 1.921	53.83 ± 1.697	38.7 ± 1.432	38.98 ± 1.692
Negative control	140.61 ± 9.847#	179.34 ± 10.69#	136.75 ± 6.72 #	157.59 ± 5.393#
NAT 250	139.51 ± 10.203#	125.87 ± 9.181**	132.79 ± 8.69#	112.59 ± 7.097**
NAT 500	140.24 ± 9.672#	122.70 ± 8.143**	136.41 ± 9.674#	109.15 ± 9.868**
NAT 750	137.70 ± 9.760#	117.77 ± 7.537**	138.67 ± 9.914#	107.65 ± 11.64**
Silymarin	136.64 ± 9.931#	112.47 ± 8.482**	142.25 ±8.111#	104.81 ± 9.18**

Results are indicated as Mean ± SEM (n = 6)

The numerical results are evaluated by application of one-way ANOVA followed by Dunnett's test for statistical significance.

Significantly different as compared to normal control ($p < 0.01$)

* Significantly different as compared to negative control ($p < 0.05$)

** Significantly different as compared to negative control ($p < 0.01$)

Table 2:

Groups	**Biochemical parameters**			
	Total Bilirubin (mg/dl)		Total Albumin (g/dl)	
	4th day	13thday	4th day	13thday
Control	0.396 ± 0.0145	0.416 ± 0.0140	5.313 ± 0.068	5.330 ± 0.078
Negative control	0.937 ± 0.0362#	2.03 ± 0.080#	3.325 ± 0.065#	2.5 ± 0.048#
NAT 250	0.888 ± 0.044#	0.598 ± 0.092**	3.321 ± 0.050#	3.925 ± 0.039**
NAT 500	0.851 ± 0.050#	0.520 ± 0.033**	3.313 ± 0.068#	4.243 ± 0.083**
NAT 750	0.915 ± 0.035#	0.496 ± 0.028**	3.298 ± 0.073#	4.350 ± 0.092**
Silymarin	0.896 ± 0.047#	0.461 ± 0.017**	3.30 ± 0.053#	4.640± 0.1056**

Results are indicated as Mean ± SEM (n = 6)
The numerical results are evaluated by application of one-way ANOVA followed by Dunnett's test for statistical significance.
Significantly different as compared to normal control ($p < 0.01$)
* Significantly different as compared to negative control ($p < 0.05$)
** Significantly different as compared to negative control ($p < 0.01$)

Table 3:

Groups	Reduced GSH μmol/mg pr	Catalase K/mg
Normal control	6.506 ± 0.0677	0.5361 ± 0.0052
Negative control	2.905 ± 0.0428#	0.249 ± 0.0067#
NAT 250	3.343 ± 0.0501**	0.263 ± 0.0081ns
NAT 500	4.158 ± 0.0490**	0.335 ± 0.005**
NAT 750	4.616 ± 0.0422**	0.359 ± 0.0044**
Silymarin	4.961 ± 0.0457**	0.396 ± 0.0059**

Results are indicated as Mean ± SEM (n = 6)
The numerical results are evaluated by application of one-way ANOVA followed by Dunnett's test for statistical significance.
Significantly different as compared to normal control ($p < 0.01$)
* Significantly different as compared to negative control ($p < 0.05$)
** Significantly different as compared to negative control ($p < 0.01$)
ns Non-significant as compared to negative control ($p > 0.05$)

Table 4:

Microscopic observations	Normal control	Negative control	NAT 250	NAT 500	NAT 750	Silymarin 100
Nuclear disintegration	-	+++	+	-	-	-
Cytoplasmic vacuolation	-	++	+	-	-	-
Centrilobular Necrosis	-	+++	-	-	-	-
Kuppfer cell hyperplasia	-	+++	++	+	-	-
Fatty infiltration	-	++	+	-	-	-
Increased cytoplasmic eosinophilia	-	+++	++	+	+	-

+++ Severe,++ Moderate,+ Mild,- None
Histopathology

Fig (a)

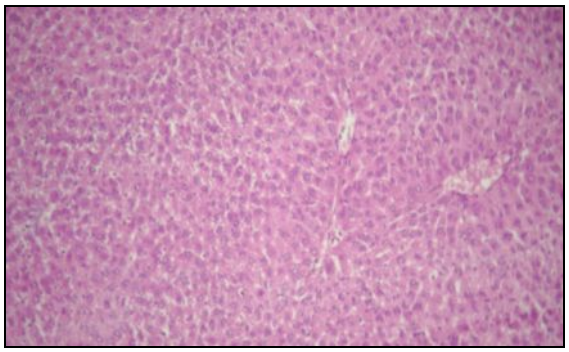


Fig (b)

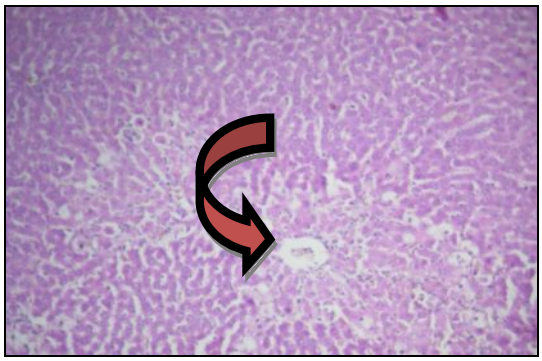


Fig (c)

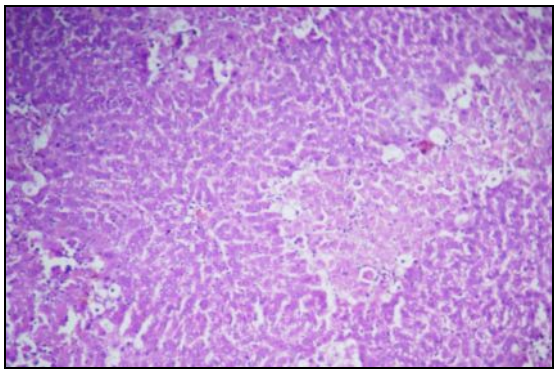


Fig (d)

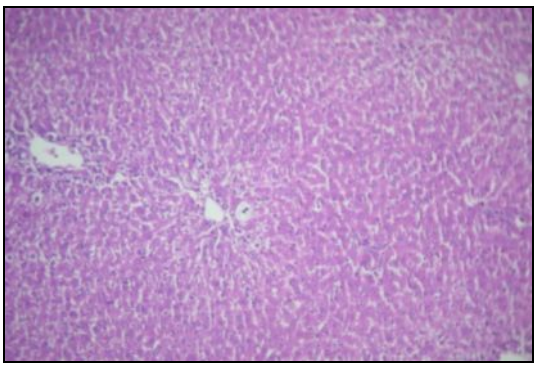


Fig (e)

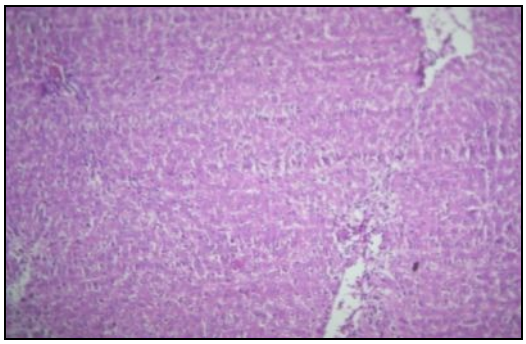
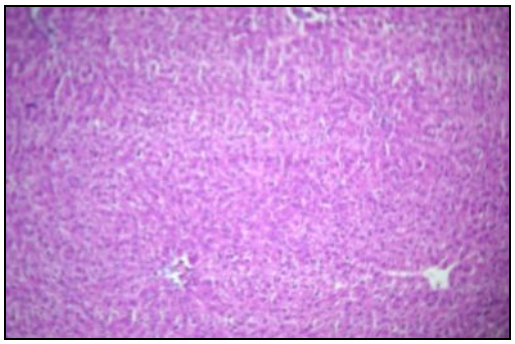


Fig (f)



Photomicrographs of liver sections taken from different treatment groups :

(a) Normal control
(b) Paracetamol control
(c) NAT 250mg/kg
(d) NAT 500mg/kg
(e) NAT 750 mg/kg
(f) Silymarin 100mg/kg

Red arrow indicates Centrilobular Necrosis.

Discussion

Aminotransferases are group of enzymes that catalyze reversible transfer of the amino acid group from an α-amino acid to an oxo acid .The largest pool of ALT is found in cytosol of hepatic parenchymal cells,whereas, AST is found in cytosol and mitochondria of

hepatocytes and also found in cardiac muscle, skeletal muscle, pancreas and kidney. Therefore, measurement of ALT is more liver specific to determine hepatocellular damage Nevertheless, AST is still being used to assess liver function since it is considered to be a sensitive indicator of mitochondria damage particularly in the centrilobular regions of liver. Treatment with NAT extract suppresses APAP induced AST and ALT elevations.[1] Recovery towards normalization of the enzymes following MNAT treatment suggested that the plant extract have some roles in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into the blood circulation. Due to treatment with MNAT extract the enzyme levels return to normal with healing of hepatic parenchyma and the regeneration of hepatocytes. In hepatopathy the degree of excretion of bilirubin from the intestine is very less and bilirubin present in the liver is excreted into the canaliculi and circulated in the bloodstream. [1,2] Hence hyperbilirubinemia is a useful index of the severity of hepatocellular dysfunction.[1,8] The treatment with MNAT counteracted the acetaminophen induced increase plasma bilirubin levels. It is known that liver synthesizes a number of proteins .Hepatotoxins impair the capacity of the liver to synthesize albumin so hypoalbuminemia is most frequent in liver diseases. Hence decline in albumin content can be deemed as a useful index of the severity of cellular dysfunction in liver diseases. The ability of MNAT to revert acetaminophen induced decline in plasma protein levels supports their hepatoprotective potential, which is a clear indication of the functional integrity of the liver. Reduced glutathione GSH is a tripeptide and plays a major role in liver detoxification.GSH is essential for maintaining the reducing capacity of the cell and the loss of this capacity may cause the cell to die.GSH can be regarded as an endogenous protective agent against hepatotoxicants. The formation of toxic reactive metabolite of NAPQI of APAP is when sulfate and glucuronide conjugation pathway become saturated-acetyl-p-benzoquinoneimine (NAPQI) conjugates rapidly with glutathione (GSH) forming mercapturic acid which subsequently is excreted in the urine.[1,2] However, excess formation of NAPQI, leads to GSH depletion which subsequently results in covalent binding of NAPQI to liver macromolecules leading to cellular necrosis.[1] Therefore, GSH is an important sulfhydryl group that maintains cellular macromolecules in functional states, serves as a key determinant of the extent of APAP induced hepatic injury. . One of the possible mechanisms for reduction in catalase activity may be due to acetaminophen induced acute liver injury, which leads to massive free radical production and saturation of catalase enzyme. MNAT possesses the capacity to scavenge free radicals produced due to acetaminophen induced hepatic damage. The normal control group of rats did not show any histological alterations in the hepatocytes. Histological sections of the liver showed similar reported changes including Centrilobular necrosis with inflammatory cell infiltration in rats treated with APAP alone. The cytochrome P450 system is abundantly distributed in Zone 3-centrilobular (Z3).[1,7] The predominant distribution of CYP450 at Z3 inflicts localized production of toxic reactive metabolites of various drugs including APAP such as N-acetyl-p-benzoquinoneimine (NAPQI).[1] This in turn induces hepatic necrosis around the Centrilobular region. Treatment with MNAT reduced the severity of histological lesions in liver and minimizes lymphocytic infiltration, nuclear disintegration and necrosis caused by acetaminophen intoxication. The possibility that MNAT extract accelerated recovery of hepatic cells was evidenced from the histopathological observation, which suggests protection against membrane fragility thus decreased the leakage of the marker enzymes into the circulation.

Conclusion

These results indicate that Methanolic extract of NAT exhibited significant hepatoregenerative potential comparable with standard silymarin by protecting against membrane fragility and by preventing the decline in glutathione level.

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References

1. S. Fakurazi, "Moringa *oleifera* Lam prevents acetaminophen induced liver injury through restoration of glutathione level", Food and Chemical Toxicology 46 (2008), 2611–2615.
2. Hui-Mei Lin, "Hepatoprotective effects of *Solanum nigrum* Linn extract against CCl4-iduced oxidative damage in rats" Chemico-Biological Interactions 171 (2008), 283–293
3. D. Sasmal, "Phcog rev.: Review Article Phytoconstituents and therapeutic potential of Nyctanthes arbortristis Linn." Pharmacognosy Reviews Vol 1, Issue 2, Jul-Dec, 2007.
4. Farnsworth N. R., Biological and phytochemical screening of plants, J Pharm Sci., 1966, 55, 225-76.
5. Ghosh M. N., Toxicity studies, Fundamental of Experimental Pharmacology, Calcutta, India, Scientific book agency, 1984, 2, 153-158.
6. Shetty A. J., Shyamjith, Deepa, Alwar M. C., Acute toxicity studies and determination of median lethal dose, Current science, 2007, 93, 917-920.
7. Ahmed O. , "The potential protective role of alpha-lipoic acid against acetaminophen-induced hepatic and renal damage" ,Toxicology 243 (2008) 261–270
8. B.P.Pimple, "Protective role of *Tamarindus Indica* against paracetamol induced hepatotoxicity in rats."Indian Journal of Pharmaceutical Sciences 69 (2007) 827-830
9. N.L. Gowri Shankar, "Hepatoprotective and antioxidant effects of *Commiphora berryi* (Arn) Engl bark extract against CCl4-induced oxidative damage in rats Food and Chemical Toxicology 46 (2008) 3182–3185.
10. Hussain Z, "Hepatoprotective activity of *Amaranthus spinosus* in experimental animals" Food and Chemical Toxicology 46 (2008) 3417–3421
11. Wu, J.-W., Lin, L.-C., Tsai, T.-H., "Drug-drug interactions of silymarin on the perspective of pharmacokinetics", Journal of Ethno pharmacology 2008 ,5299.

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